Amyloid P Component Binds to Keratin Bodies in Human Skin and to Isolated Keratin Filament Aggregates In Vitro

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Dermal keratin bodies, consisting mainly of keratin intermediate filament aggregates (KIFA) coated with IgM anti-KIF autoantibodies, are present in normal human skin and occur in increased quantities in certain skin diseases. Keratin bodies are normally rapidly removed, but in primary localized cutaneous amyloidosis (PLCA) they are converted by an unknown mechanism to amyloid. Amyloid P component (AP), a glycoprotein identical to, and derived from, the normal plasma protein serum amyloid P component (SAP), is present in all forms of amyloid including PLCA. We investigated the interaction between SAP, keratin bodies, and KIFA. Immunofluorescence staining of normal skin using fluoresceinated anti-SAP and rhodamine-conjugated anti-IgM, or AE-1/AE-3 anti-keratin antibodies followed by Texas Red-conjugated anti-mouse immunoglobulin, showed that 52% ± 4 (mean ± sem, n = 6) of keratin bodies bound anti-SAP. Similar findings were present in a biopsy from a patient with lichen planus. Isolated KIFA, prepared by 8M urea extraction of normal human epidermis or cultured keratinocytes, were preincubated with normal human serum as a source of SAP and then stained with fluoresceinated anti-SAP. Bright fluorescence seen when the incubation medium contained Ca^{++} was absent in the presence of ethylenediamine tetraacetic acid. Specific Ca^{++}-dependent binding of SAP to KIFA was confirmed using immunoblotting. Binding of SAP to KIFA did not prevent their degradation following exposure to trypsin or alpha-chymotrypsin. Similarly, partial enzymatic digestion of KIFA did not abrogate their ability to bind SAP. Our findings, that SAP is associated with keratin bodies in skin and exhibits Ca^{++}-dependent binding to KIFA in vitro, identify keratin filaments as a newly recognized ligand for SAP. *J Invest Dermatol 91:22–28, 1988*

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Abbreviations:
- AP: amyloid P component
- EDTA: ethylenediamine tetraacetic acid
- FITC: fluorescein isothiocyanate
- HRP-protein A: horseradish peroxidase-conjugated staphylococcal protein A
- KIF: keratin intermediate filament(s)
- KIFA: keratin intermediate filament aggregates
- PAGE: polyacrylamide gel electrophoresis
- PBS: phosphate buffered saline
- PLCA: primary localized cutaneous amyloidosis
- PUVA: psoralen plus ultraviolet A
- RITC: rhodamine-conjugated
- SAP: serum amyloid P component
- SDS: sodium dodecyl sulfate
- TR: Texas Red

We have recently demonstrated that apoptosis, whereby keratinocytes undergo filamentous degeneration with extrusion of keratin (cytoid, colloid, Civatte) bodies composed chiefly of keratin intermediate filament aggregates (KIFA) into the upper dermis, occurs as a ubiquitous physiologic process in normal human skin [1]. Increased keratin body formation as a result of keratinocyte destruction is a marked histologic feature of a number of important lymphocyte-mediated skin diseases including lichen planus, lupus erythematosus, certain drug eruptions, and cutaneous graft-versus-host disease. This finding may account for the presence of anti-KIF autoantibodies in human serum, and the regular association of IgM with keratin bodies in vivo may represent specific binding of such anti-KIF autoantibodies to KIFA [1–5]. Keratin bodies in normal skin and in lymphocyte-mediated skin diseases are thought to be routinely eliminated, following opsonization by anti-KIF autoantibodies, by phagocytosis and/or enzymatic digestion by macrophages and dermal fibroblasts [6,7]. Filamentous degeneration of keratinocytes, resulting in the formation of numerous keratin bodies, is also a cardinal feature of the histopathology of primary localized cutaneous amyloidosis (PLCA) [8–11]. Histochemical, immunohistochemical, and ultrastructural studies have suggested that the amyloid deposits found in PLCA, and in certain skin neoplasms and following psoralen plus ultraviolet A (PUVA) therapy, are derived from such keratin bodies and are composed in part of KIF material [8–16]. It has been proposed that in PLCA defective phagocytosis of keratin bodies results in their retention in the dermis for prolonged periods of time, thereby favoring transformation of KIF material into amyloid by some as yet unknown mechanism, perhaps involving enzymatic digestion [8,9,11,17]. Enzymatic digestion in vitro of certain Bence Jones proteins (the precursor proteins of amyloid fibrils in myeloma-associated systemic amyloidosis) yields amyloid-like fibrils [18], and our preliminary experiments involving enzymatic digestion of KIF in vitro have also produced fibrils that resemble amyloid ultrastructurally [5].

Amyloid P component (AP) is a glycoprotein which is found in deposits of all forms of amyloid, including cutaneous amyloid [17,19–21], and which is indistinguishable from, and derived from, the normal plasma glycoprotein serum amyloid P component (SAP) [19,22]. SAP is composed of 10 identical glycosylated polypeptide subunits, each with an approximate molecular weight of 25,000 daltons, arranged with cyclic pentameric symmetry in a disc-like configuration. SAP closely resembles CRP, the classical acute phase reactant, in terms of molecular configuration, ultrastructural appearance, and amino acid sequence, but is otherwise unrelated to any known protein [19]. SAP and CRP together form a distinct family of proteins of common evolutionary origin termed pentraxins. SAP may be extracted from normal human serum by virtue of its Ca^{++}-dependent binding to agarose [23]. SAP exhibits Ca^{++}-dependent binding to a variety of other ligands, including fibronectin, C4 binding protein, glycosaminoglycans, and isolated amyloid fibrils in vitro [24–27]. Interestingly, sulfated glycosaminoglycans, like AP, are associated with deposits of all forms of amyloid [28]. SAP is also a normal matrix glycoprotein of glomerular basement membrane, in which it is covalently linked to collagen and/or other matrix proteins [29]. We have shown that SAP is a constituent of the microfibrillar component of elastic tissue fibres in normal human skin and other tissues [20,30,31]. AP on elastic fibres, via an interaction with tissue fibronectin [24,25] and glycosaminoglycans [26], may play an important role in the maintenance of connective tissue architecture [17]. The significance of amyloid P component in the pathogenesis of amyloidosis is unknown, but it has been suggested that it may promote the deposition or inhibit the degradation of amyloid fibrils [17,32]. We have therefore studied the interaction between AP/SAP, keratin bodies, and isolated KIFA in order to investigate a potential role for AP in the pathogenesis of PLCA. We report here that SAP is associated with keratin bodies in vivo and exhibits Ca^{++}-dependent binding to isolated KIFA in vitro, but that such binding does not appear to protect KIFA against enzymatic degradation under the conditions studied.

MATERIALS AND METHODS

Normal human serum was obtained from volunteers and from pooled test samples provided by the National Blood Transfusion Service. Normal human skin specimens were obtained at surgical operations, and lesional skin was obtained at the time of diagnostic biopsy. Informed consent was obtained from all individuals.

Preparation of SAP SAP was isolated from normal human serum as previously described [23,33].

Antisera Antihuman SAP antibodies were raised by immunization of sheep and rabbits with isolated pure SAP as described [23,33]. The IgG fraction of the sheep antihuman SAP obtained by DEAE-cellulose chromatography was conjugated with fluorescein isothiocyanate (FITC), and the IgG fraction of rabbit anti-human SAP obtained by staphylococcal protein A absorption was used in immunoblotting studies. Rhodamine-conjugated (RITC) anti-human IgM (μ chain specific) was obtained from Dako Immunoglobulins (Copenhagen, Denmark), monoclonal antikeratin antibodies AE-1/AE-3 [34] from Hybritech Inc. (San Diego, CA), and Texas Red (TR)-conjugated sheep anti-mouse immunoglobulin from Amersham International (Amersham, UK).

Immunofluorescence Staining of Skin Biopsies Six micrometer vertical cryostat sections of normal and lesional skin specimens were stained by a double immunofluorescence technique with FITC-anti-SAP diluted 1:50 in phosphate buffered saline (PBS) and either RITC-anti-IgM (dilution 1:20 in PBS) or AE-1/AE-3 antikeratin (dilution 1:20 in PBS containing 1% bovine serum albumin) followed after washing with PBS by TR-anti-mouse immunoglobulin, to identify keratin bodies. Sections were examined using a Leitz Dialux EB22 microscope equipped with epifluorescence. At least 25 IgM-positive keratin bodies in nonconsecutive serial sections from each biopsy were investigated for binding of anti-SAP by two independent observers. The percentage of keratin bodies that were positive for SAP was then determined by taking a mean of the values obtained by each observer.

Extraction of Epidermal Proteins After separation from the dermis by incubation with 0.02M ethylenediamine tetraacetic acid (EDTA) buffer, minced epidermis was boiled for 20 min in buffer containing 0.0625M Tris-HCL pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.01% Bromophenol Blue, and 1% Triton X-100, to yield an extract of proteins from whole epidermis [3].

Preparation of Isolated KIF Aggregates KIFA were prepared from normal human epidermis or cultured human keratinocytes grown by a modification of the Rheinwald and Green technique [35], as previously described [7]. In brief, following separation from the dermis by incubation with 0.02M EDTA buffer, minced epidermis was extracted in an 8M urea solution containing 0.01M Tris-HCl (pH 9.0) and 0.1M 2-mercaptoethanol for 4 h at room temperature. The cellular debris was removed by centrifugation at 10,000 rpm for 30 min and the supernatant was dialyzed against 1,000 volumes of PBS overnight at room temperature, resulting in the formation of a suspension of KIFA. Further purification was achieved by redissolving the KIFA in urea solution and repeating the procedure.

KIFA extracted from normal human epidermis consisted of five major proteins, the molecular weights of which (50, 56.5, 58, and 65–67 kd) were as previously described by us [2], and in accordance with findings reported by other authors [36]. KIFA prepared from cultured human keratinocytes consisted of four proteins of molecular weight 48, 50, 56, and 58 kd, which reacted with AE-1/AE-3 monoclonal antikeratin antibodies on immunoblotting and gave peptide mapping patterns similar to those reported by other authors [37] (data not shown).

Reaction of KIF Aggregates with SAP In order to determine whether SAP binds to KIFA, initial experiments were carried out under conditions previously shown to favor binding of SAP to isolated amyloid fibrils [27]. Ten milligrams of KIFA suspension were incubated with 1.5 ml of normal human serum as a source of SAP, with constant agitation by end over end tumbling for 2 h at room temperature. The KIFA were then washed twice for 15 min with either "calcium buffer" (0.01M Tris, 0.138M NaCL, 0.002M $CaCl_2$, pH 8.0) or "EDTA" buffer (0.01M Tris, 0.138M NaCL, 0.01M EDTA, pH 8.0), followed by an overnight wash at 4°C with the respective buffer. In a separate experiment, KIFA were incubated with 50 ml of pooled human serum, to ensure a large excess of SAP and saturation of any potential binding sites on KIFA.

The presence or absence of binding of SAP to KIFA after washing in calcium or EDTA buffer was then determined by an immunofluorescence technique as follows. One drop of the KIFA-SAP reaction product was air-dried on a glass slide and incubated with FITC-anti-SAP in calcium buffer or EDTA buffer (dilution 1:50) for 30 min and then washed extensively with the appropriate buffer. In other experiments, drops of KIFA suspension were first air-dried on glass slides before incubation with (a) normal human serum, (b) normal human serum dialyzed against calcium buffer for 4 hours at 4°C, or (c) pure SAP in calcium buffer (18 μg/ 30 μl), before extensive washing in calcium buffer or EDTA buffer as above. Slides were examined using a Leitz Dialux EB22 fluorescence microscope. Binding of SAP to KIF was also investigated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting as below.

Enzymatic Digestion of KIF Aggregates KIFA (from either normal human epidermis or from cultured keratinocytes) were offered for enzymatic digestion following preincubation with a large excess of normal human serum as a source of SAP and extensive washing with either calcium buffer or EDTA buffer, as above. Trypsin (from bovine pancreas, 2,861 U/mg) was obtained from US Biochemical Co. (Cleveland, OH) and alpha-chymotrypsin (from bovine pancreas, type I–S, 60 U/mg) was obtained from Sigma Chemical Co. (St. Louis, MO). The optimal concentrations and reaction times for enzymatic digestion were determined in initial experiments using a range of from 0.25 to 5 μg of enzyme, and a range of incubation durations of from 1 to 60 min. For the definitive experiments, 100 μl samples of a 10 mg/ml suspension of KIFA preincubated with normal human serum as a source of SAP, and washed in either calcium buffer or EDTA buffer, were then incubated at room temperature with the enzymes under optimal concentration-time conditions, which were 1 μg/ 10 μL calcium buffer for 20 min for trypsin, and 0.5 μg/ 10 μL calcium buffer for 10 min for alpha chymotrypsin. At the end of the incubation period, enzyme activity was inhibited by addition of Laemmli sample buffer containing 5% 2-mercaptoethanol [5]. The effect of exposure to SAP, in the presence or absence of calcium, on the degree of enzymatic degradation of KIFA was monitored by SDS-PAGE and immunoblotting as below.

In addition, the effect of enzymatic digestion of KIFA on their subsequent ability to bind SAP was investigated. Two milligrams of KIFA suspension were incubated with 1 μg/ 10 μL trypsin at room temperature. Aliqots of the reaction mixture were withdrawn at the following time intervals: 1, 5, 10, 15, 30, 60, 120, 240, and 360 min. Further enzymatic digestion of KIFA in the samples was prevented by inactivation of trypsin by addition of 2.5 μg trypsin inhibitor (Sigma), and by washing with PBS. The degree of digestion of the KIFA at each time interval was monitored by SDS-PAGE. Samples of KIFA exposed to the enzymes for varying durations were reacted with normal human serum, washed extensively with calcium buffer, and stained with anti-SAP by an immunofluorescence method as above.

In vitro reconstituted KIFA, and material derived following partial enzymatic digestion of KIFA, were air-dried on glass slides, fixed with 10% formalin for 10 min, and examined for thioflavine T fluorescence or green birefringence under polarized light after staining with alkaline Congo red dye.

Polyacrylamide Gel Electrophoresis and Immunoblotting Samples of proteins extracted from whole epidermis, KIFA suspensions reacted with normal human serum and then washed extensively with either calcium buffer or EDTA buffer, purified human SAP, KIFA proteins following enzymatic digestion, and KIFA reacted with normal human serum, washed extensively with either calcium buffer or EDTA buffer, and then exposed to trypsin or alpha-chymotrypsin under optimal concentration-duration conditions, were analyzed by SDS-PAGE using 10.5% gels and immunoblotting as described [2,5]. Purified SAP was also analyzed by SDS-PAGE using a 12% and a 15% gel, and peptides obtained after enzymatic digestion of KIFA were analyzed using a 15% gel. In the immunoblot procedure, transferred proteins were first stained with fast green FCF (Polysciences Inc., Warrington, PA), and their position on the nitrocellulose paper was marked by punching with a

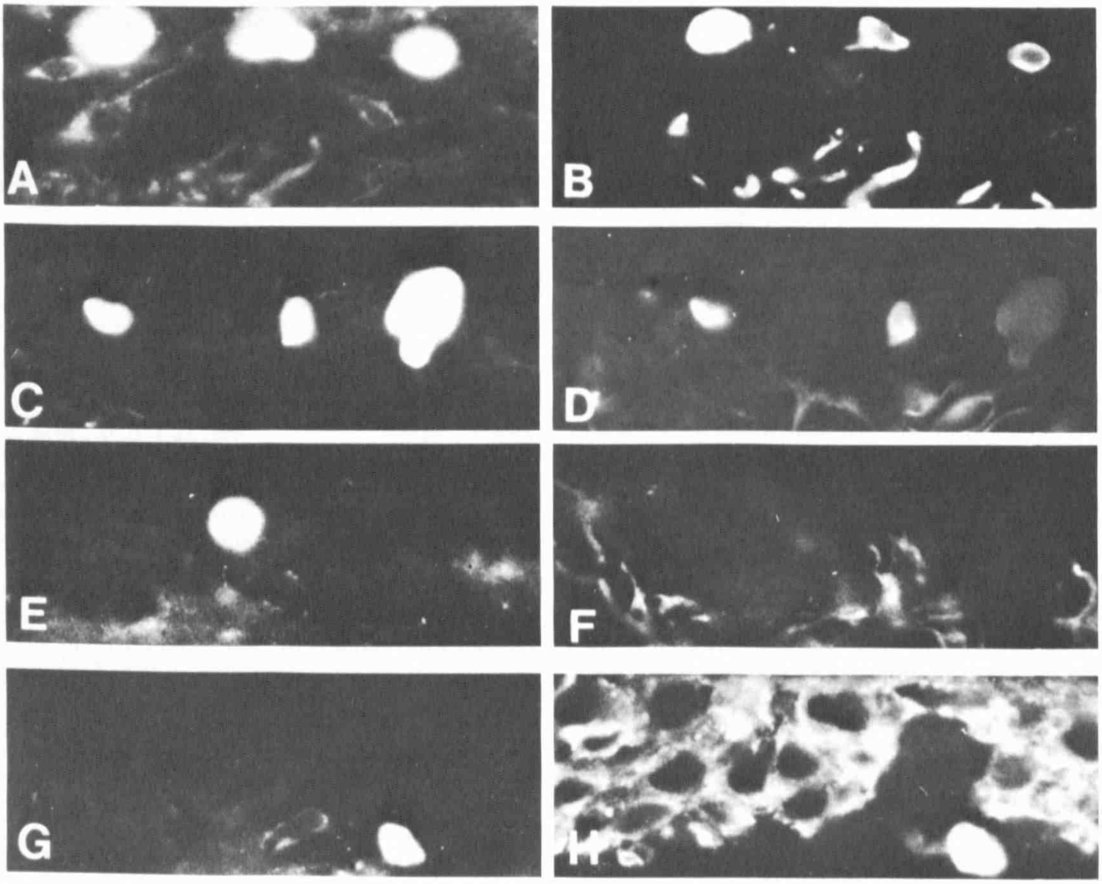

Figure 1. Double immunofluorescence staining of keratin bodies in vertical cryostat sections of normal human skin with FITC anti-SAP and either RITC-anti-IgM or AE-1/AE-3 antikeratin antibodies followed by TR-anti-mouse immunoglobulin. A large proportion of keratin bodies (brightly fluorescent globular structures situated in the uppermost papillary dermis just beneath the dermo-epidermal junction) that stain strongly with RITC-anti-IgM *(A,C,E)* or anti-keratin antibodies *(H)* also fluoresce brightly with FITC-anti-SAP *(B,D,G)*. Keratin bodies occasionally show peripheral accentuation of staining with FITC-anti-SAP *(B)*. Other keratin bodies show only weak fluorescence *(D)* or do not stain at all *(F)* with FITC anti-SAP. The subepidermal location of a globular keratin body relative to the lower epidermis, stained with anti-keratin antibodies, is clearly seen *(H)*. × 640

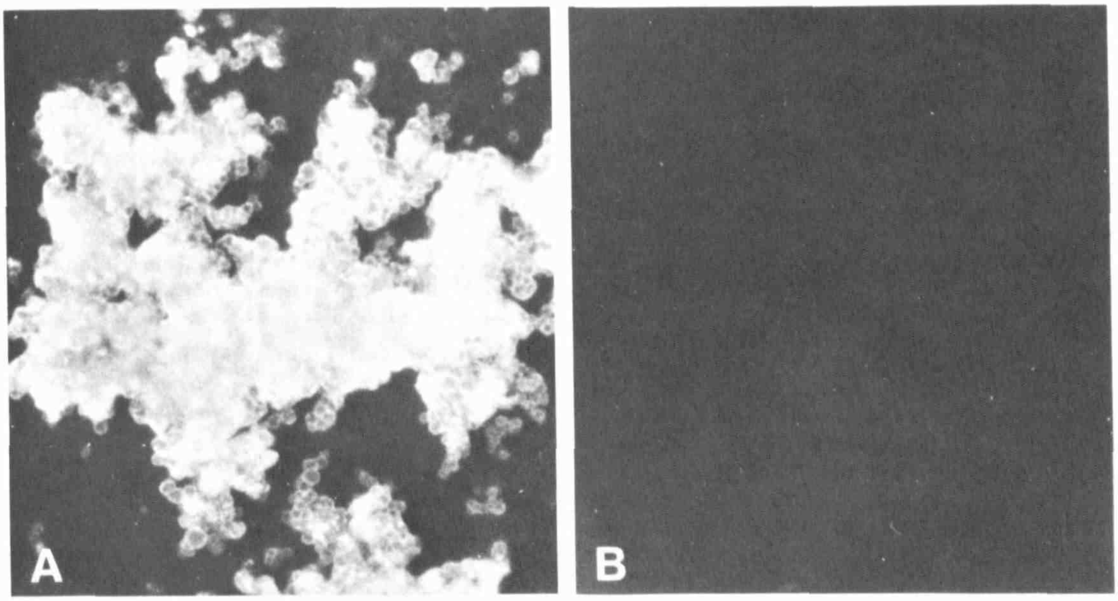

Figure 2. Bright fluorescence with FITC-anti-SAP of KIFA incubated with normal human serum and washed with calcium buffer *(A)* is absent when washes are carried out with EDTA buffer *(B)*. × 520

needle [3]. SAP was then detected using rabbit anti-human SAP (dilution 1 : 500) followed by horseradish peroxidase-conjugated staphylococcal protein A (HRP-protein A) (Kirkegaard and Perry Labs, Gaithersburg, MD) at a dilution of 1 : 200 [2].

RESULTS

SAP is Associated with Keratin Bodies In Vivo Since keratin bodies regularly bind IgM anti-KIF autoantibodies in vivo, they may be identified in tissue sections by immunofluorescence using anti-human IgM antibodies [1]. Double immunofluorescence staining of cryostat sections of normal human skin from six individuals with FITC-anti-SAP and RITC-anti-IgM demonstrated that 52% ± 4 (mean ± sem) of IgM positive keratin bodies also stained positively for SAP, with a range of between 41% and 59%. The intensity of fluorescence with anti-IgM was uniformly strong, whereas there was considerable variation in the intensity of staining of individual SAP positive keratin bodies with anti-SAP; staining with anti-SAP was occasionally accentuated at the periphery of the keratin bodies (Fig 1*A–D*). Although many keratin bodies were positive for SAP, approximately half of them failed to stain with anti-SAP (Fig 1*E, F*). The association of SAP with keratin bodies in normal human skin was confirmed in further double immunofluo-

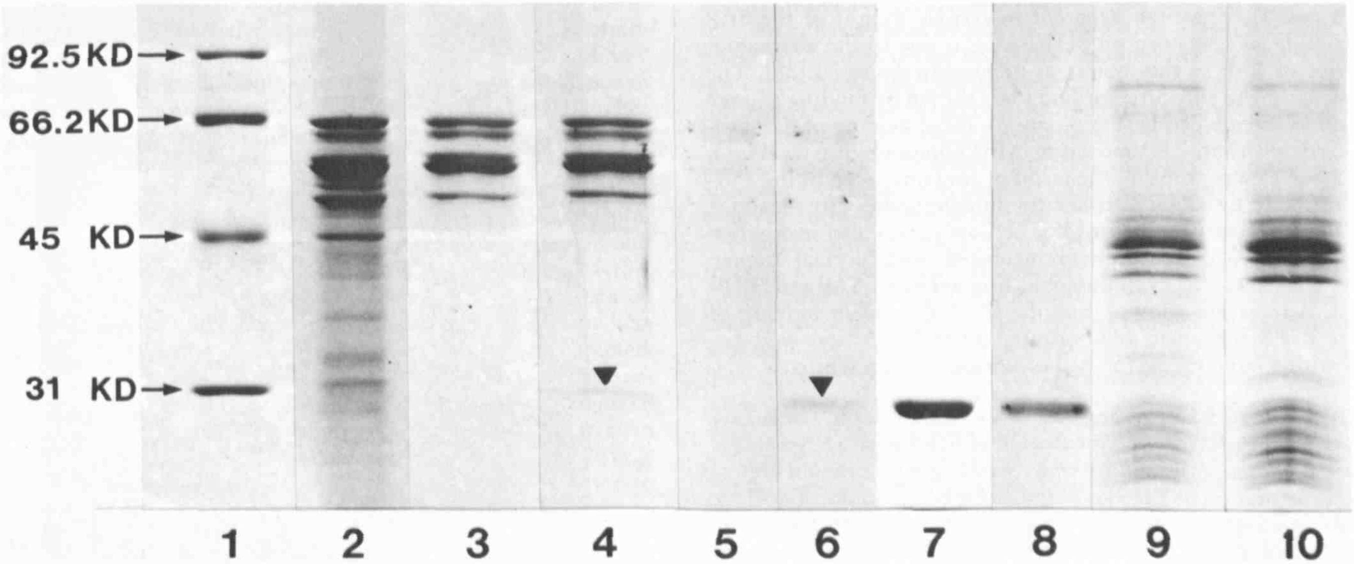

Figure 3. Analysis of interaction of SAP with KIFA using SDS-PAGE and immunoblotting with anti-SAP. *Lane 1:* molecular weight markers; *lane 2:* SDS-PAGE of proteins extracted from whole epidermis; *lane 3:* SDS-PAGE of KIFA reacted with normal human serum and washed with EDTA buffer; *lane 4:* SDS-PAGE of KIFA reacted with normal human serum and washed with calcium buffer (*arrowhead* identifies extra band); *lane 5:* immunoblotting with anti-SAP of KIFA reacted with normal human serum and washed with EDTA buffer; *lane 6:* immunoblotting with anti-SAP of KIFA reacted with normal human serum and washed with calcium buffer (*arrowhead* denotes extra band identified as SAP), *lane 7:* SDS-PAGE of 1 μg of pure SAP; *lane 8:* immunoblotting with anti-SAP of pure SAP; *lane 9:* SDS-PAGE of KIFA preincubated with normal human serum, washed with EDTA buffer, and reacted with alpha chymotrypsin; *lane 10:* SDS-PAGE of KIFA preincubated with normal human serum, washed with calcium buffer, and reacted with alpha chymotrypsin.

rescence studies in which keratin bodies were identified using monoclonal antibodies AE-1/AE-3 (Fig 1*G, H*). In several specimens of normal human skin, corpuscles the size of keratin bodies or larger situated in the upper reticular dermis fluoresced brightly with anti-SAP, but did not stain with either anti-IgM or AE-1/AE-3 antibodies. These corpuscles often had a facetted, angular, or polygonal outline, and staining with anti-SAP was accentuated around their rim. It is likely that these structures represent elastic globes.

Similar findings with regard to positive staining of keratin bodies with anti-SAP were present in a skin biopsy taken from a patient with lichen planus of the scalp and scarring alopecia.

SAP Exhibits Ca^{++}-Dependent Binding to Isolated KIF Aggregates In Vitro KIFA in suspension, from both normal human epidermis and from keratinocyte cultures, showed bright fluorescence with anti-SAP following incubation with normal human serum as a source of SAP, provided all subsequent washes were carried out using calcium buffer (Fig 2*A*). Staining was absent when washes were carried out using EDTA buffer (Fig 2*B*). Specific Ca^{++}-dependent binding of SAP to KIFA was also seen when KIFA that had been air-dried on slides were stained with anti-SAP following incubation with normal human serum, normal human serum dialyzed against calcium buffer, or pure SAP in calcium buffer, provided all subsequent washes were carried out using calcium buffer. Once again, fluorescence was absent if washes were carried out with EDTA buffer.

The nature of epidermal keratin proteins and the interaction of SAP with KIFA as analyzed using SDS-PAGE and immunoblotting with anti-SAP, are shown in Fig 3. Analysis of the proteins present in an extract of whole human epidermis revealed a large number of bands (Fig 3, *lane 2*). SDS-PAGE of purified KIFA, reacted with 1.5 ml of normal human serum and washed with EDTA buffer, revealed the 5 recognized KIF protein bands only (Fig 3, *lane 3*). By contrast, SDS-PAGE of KIFA reacted with normal human serum and washed with calcium buffer identified an additional protein band with an apparent molecular weight of about 31 kd (Fig 3, *lane 4*). Immunoblotting with anti-SAP identified this extra protein band as SAP (Fig 3, *lanes 5* and *6*). The identity with SAP of the additional protein band in preparations of KIFA reacted with normal human serum and washed with calcium buffer was confirmed by its co-migration with pure SAP on SDS-PAGE (Fig 3, *lane 7*). The high apparent molecular weight of the SAP polypeptide subunit seen on SDS-PAGE is due to the anomalous behavior of SAP in this system as previously discussed [38]. The specificity of the anti-SAP antibody was shown by binding to pure SAP on immunoblotting (Fig 3, *lane 8*). Faint staining of KIF protein bands with anti-SAP antiserum in the immunoblot assay (Fig 3, *lanes 5 and 6*) was probably the result of contamination of the anti-SAP antibody by rabbit anti-keratin autoantibodies cross-reactive with human keratin proteins [39], because it was also seen when KIFA, which had not been preincubated with normal human serum, were analyzed by immunoblotting with anti-SAP and HRP-protein A (data not shown). Specific Ca^{++}-dependent binding of SAP to KIFA was again demonstrated when KIFA were preincubated with a large excess (50 ml) of normal human serum.

SAP Binding to KIF Aggregates Does Not Prevent Their Enzymatic Degradation Preincubation of KIFA with a large excess of normal human serum followed by washing with calcium buffer, to ensure saturation of KIF binding sites for SAP, did not alter their subsequent enzymatic degradation on incubation with trypsin or alpha-chymotrypsin; the appearances on SDS-PAGE of the digested KIF material did not differ significantly from those seen if KIFA preincubated with normal human serum were washed with EDTA buffer, which elutes off SAP bound to KIF (Fig 3, *lanes 9* and *10*). Binding of SAP to the KIFA washed with calcium buffer before the digestion procedure was confirmed by immunofluorescence. Exposure to enzymatic digestion did not alter the antigenicity of SAP, because it was still detectable with anti-SAP in immunoblotting studies (data not shown).

SAP Binds to Enzymatically Degraded KIF Aggregates Enzymatic digestion with trypsin of native KIFA did not abrogate their subsequent ability to bind SAP as determined by immunofluorescence, following preincubation with normal human serum and washing in calcium buffer. Bright fluorescence of KIF material with anti-SAP was seen even after 6 h of digestion (Fig 4). At this time interval, the discrete globular appearance of native KIFA stained with anti-SAP was replaced by a granular pattern of fluorescence. Binding of SAP occurred despite extensive degradation of KIF proteins as evidenced by SDS-PAGE (Fig 5); the molecular weight of the SAP-binding polypeptides ranged mainly between 12.2 and 22 kd after 6 h of digestion.

Congo Red and Thioflavine T Staining of Native and Digested KIF Aggregates Neither native KIFA, nor KIF material produced following trypsin digestion of KIF aggregates, showed Thioflavine T fluorescence or green birefringence under polarized light after alkaline Congo Red staining.

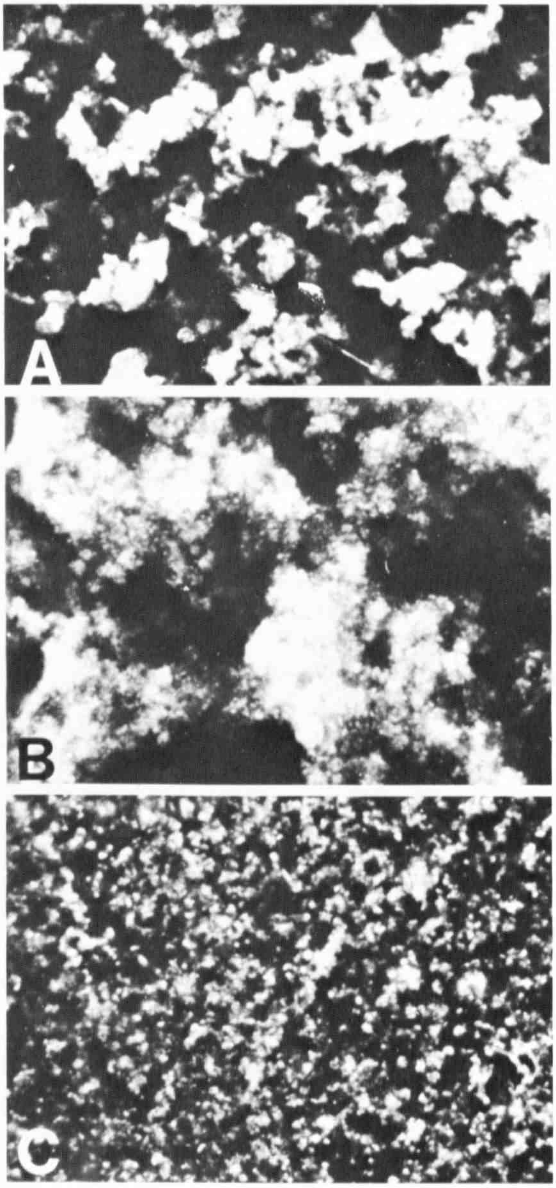

Figure 4. Undigested KIFA *(A)* and KIFA digested with trypsin for 30 min *(B)* and 6 h *(C)* were incubated with normal human serum followed by FITC-anti-SAP; all fluoresce brightly, but the globular staining pattern seen with undigested KIFA is progressively replaced following digestion by a granular appearance × 480.

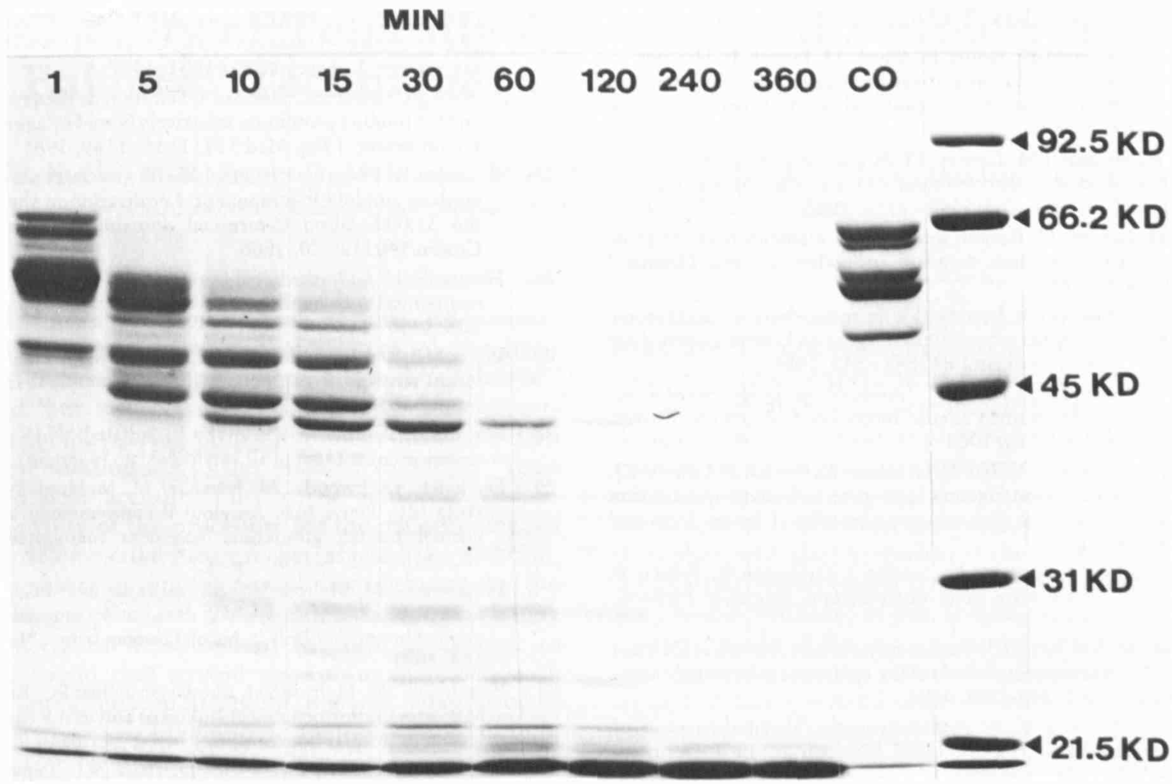

Figure 5. SDS-PAGE time lapse analysis of the digestion of KIFA with trypsin shows progressive generation of low molecular weight KIF protein degradation products. (*CO*: control undigested KIFA). *Right hand lane*: molecular weight standards.

DISCUSSION

The constant association of AP with amyloid deposits, including those of PLCA, has led to the suggestion that AP may be of importance in the deposition and persistence of amyloid material [17,32]. Because the amyloid deposits in PLCA are thought to be derived at least in part from keratin bodies composed principally of KIFA [8-16], we investigated the interactions between SAP, keratin bodies, and KIFA. Our results demonstrate in vivo binding of SAP to about half of the keratin bodies present in the papillary dermis of normal human skin, and to a similar proportion of the keratin bodies in a case of lichen planus of the scalp (Fig 1). We have also clearly shown, using immunofluorescence, SDS-PAGE and immunoblotting techniques, that SAP from normal human serum, as well as isolated, purified SAP, binds in a specific Ca^{++}-dependent fashion in vitro to isolated KIFA prepared from normal human skin and from cultured keratinocytes (Figs 2 and 3). We investigated KIFA derived from keratinocyte cultures, in addition to KIFA prepared from whole normal human epidermis, because KIF in cultured keratinocytes and in keratin bodies display identical staining characteristics with defined monoclonal antikeratin antibodies [16]. We surmise from our findings that KIF represent a newly recognized ligand for SAP. It is not clear why only about half of the keratin bodies in normal and lesional skin are associated with AP/SAP. It may be that newly generated keratin bodies fail for some reason to bind AP/SAP, or that mature keratin bodies lose their capacity to bind AP/SAP. The latter alternative seems, however, unlikely, because we report here that the binding sites on KIFA for SAP remain intact even after extensive enzymatic degradation of KIFA (Figs 4 and 5).

It has recently been suggested that the Ca^{++}-dependent binding of SAP to amyloid A protein (the amyloid fibril protein present in reactive secondary systemic amyloidosis) may involve a small glycine rich segment of the molecule [40]. KIF are very rich in glycine residues [41,42], which are mainly situated in the nonhelical end domains and are potentially available as binding sites. The association of SAP with KIFA and with keratin bodies may therefore occur as a result of binding of SAP to these glycine rich sites. However, SAP has recently been reported to show Ca^{++}-dependent binding to glycosaminoglycans [26]. Glycosaminoglycans are synthesized by keratinocytes [43] and are a normal constituent of the dermo-epidermal basement membrane. Keratin bodies and amyloid deposits of PLCA are derived from filamentous degeneration of keratinocytes, may contain basement membrane zone material [44], and stain positively with the periodic acid-Schiff method. Although PLCA has not been investigated for the presence of glycosaminoglycans yet, sulfated glycosaminoglycans have always been associated with amyloid deposits in all forms of amyloid in which they have been sought [28]. We cannot, therefore, entirely exclude the possibility that apparent SAP binding to keratin bodies occurs as a result of binding to glycosaminoglycans at present.

Neither undigested KIFA nor KIFA enzymatically digested with trypsin, fluoresced with thioflavine T, or stained with alkaline Congo Red. Enzymatic digestion with trypsin alone is therefore insufficient to generate amyloid material from KIFA, and the mechanism by which keratin bodies are transformed to amyloid material in PLCA remains unknown. The significance of the constant association of SAP with amyloid deposits, including those of PLCA, also remains uncertain. With regard to PLCA, it has been suggested that persistence of keratin bodies may in some way favor their conversion to amyloid. Following our discovery that SAP binds to keratin bodies and to KIFA, we investigated the effect of SAP on the digestion of KIFA by proteinases. We found that SAP binding to KIFA did not protect them from enzymatic degradation on incubation with either trypsin or alpha-chymotrypsin (Fig 3). This fact, together with our finding that SAP binds to keratin bodies in normal and lesional human skin in the absence of amyloid deposition, could be interpreted as suggesting that AP does not play an active role in amyloidogenesis. Alternatively, our failure to demonstrate a function for SAP in protection of KIFA against enzymatic digestion may be because our in vitro model system does not adequately simulate the conditions operating in vivo.

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